

Amendments to the Drawings

The attached sheets of drawings include changes to FIGS. 1-11 as needed to answer the issues raised on form PTO 948. These sheets replace all the original sheets of drawings.

Attachment: Replacement sheets

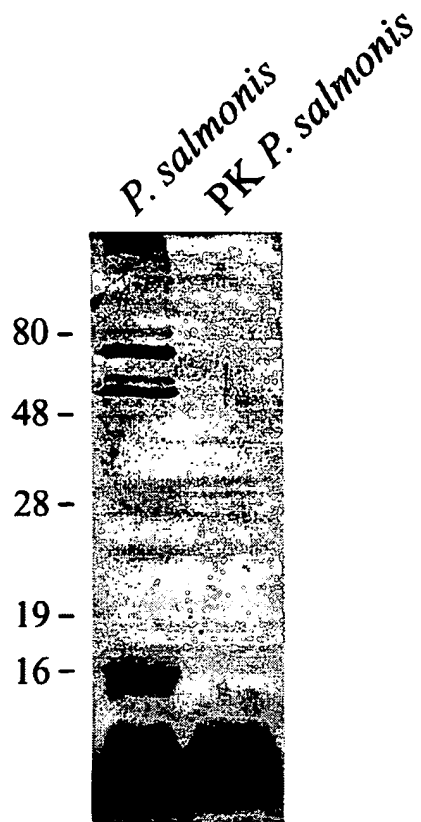
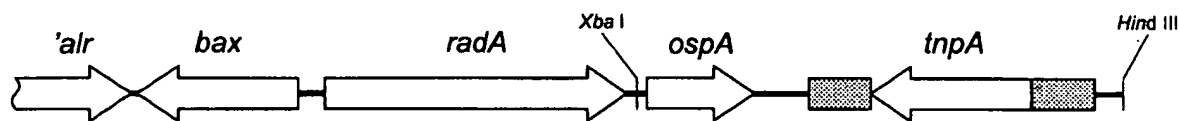
FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*

FIGURE 2

A. ORF's in the region of the *ospA* gene from *P. salmonis*



B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGAAGGTAGTAGTCTAATTATTATCAGTGTGTTTTAGTTGGCTGTGCCCAGA
 ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCGGTGTTGCTGGCCAGCTGTTTGG
 TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTAAATTGGTTCTAAAATC
 GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGAAAAGGTAAAAGCAGGGCAAG
 TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTTGAGCCAGTGCGTACTTACCAGCGTTA
 CAATAAGCAAGAGCGTCGCCAGCAATATTGTGAGAATTTACAGAAAAGGCGATGATTGCAGGGCAGAAG
 CAAGAGATTTACGGCACTGCATGCCGGCAACCGGATGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Amino acid sequence of OspA protein (SEQ ID:2)

MNRGCLQGSSLIISVFLVGCANFSRQEVGAATGAVVGGVAGQLFGKSGRVAMAIGGAVLGGLIGSKI
 QSMDQQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQYCREFOQKAMIAGQK
 QEIYGTACRQPDGRWQVISTEK

C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

	10	20	30	40	
<i>P. salmonis</i> :	MNRGCLQGS	SLIHSV---	FFVGCA---	QNF	
<i>R. prowazekii</i> :	MKLLSKIMT	IALAASML	QACNGQ	SGM	
	10	20	30	40	
	50	60	70	80	90
<i>P. salmonis</i> :	FGKCS	SRV	AMAI	GGAVL	GGLIG
<i>R. prowazekii</i> :	FGCKG	QGLV	GVGV	GAILG	AVLGG
	50	60	70	80	90
	100	110	120	130	140
<i>P. salmonis</i> :	GQVTR	WRNP	DTG	NSYS	VEPV
<i>R. prowazekii</i> :	GSNID	WRNP	DGN	HGVV	TPNK
	100	110	120	130	140
	150	160			
<i>P. salmonis</i> :	ET	YGTAC	RQPD	GRWQ	VISTEK
<i>R. prowazekii</i> :	KTYG	NACRQ	PDGRWQ	VV	
	150				

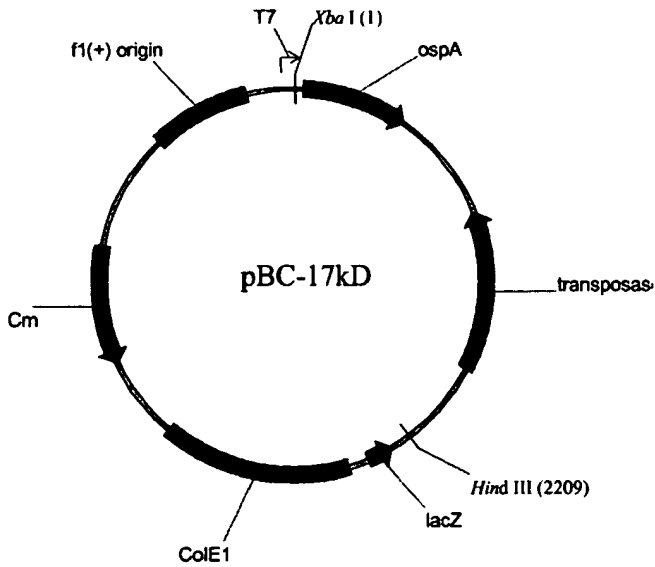
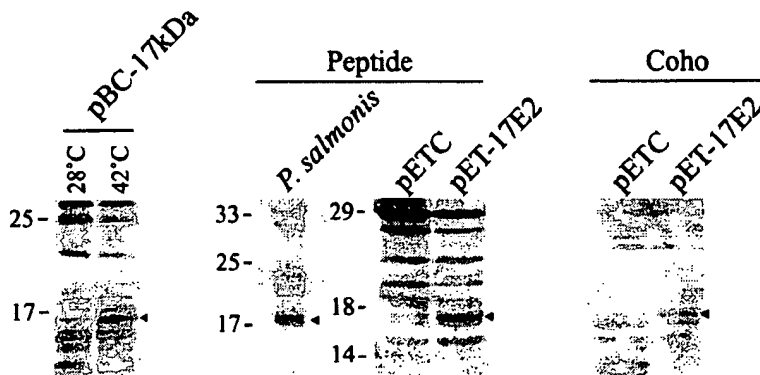
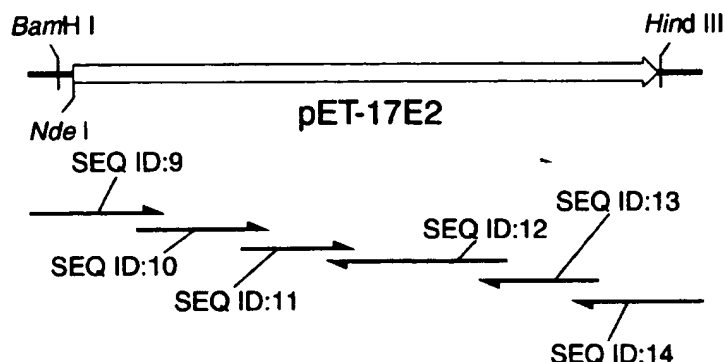
FIGURE 3**A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.****B. Western blot analysis of OspA expression.**

FIGURE 4.**A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.****B. Oligonucleotide #1 (SEQ ID:9)**

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC
ATTATCTCTGTTTTCCCTGGTGGGTTGCGCCAGAACTTCAG

Oligonucleotide #2 (SEQ ID:10)

TGGGTTGCGCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC
CGGCCAGCTGTTTCGGTAAAGGCTCTGGTCGTGTGGCGATG

Oligonucleotide #3 (SEQ ID:11)

AAAGGCTCTGGTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG
GTCAGAGCATGGACCAGCAGGATA

Oligonucleotide #4 (SEQ ID:12)

GTTCCACAGAGTAGCTGTTACCGGTGTCCGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTTC
CAGAGACTGGTTCAGTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

Oligonucleotide #5 (SEQ ID:13)

GGTGCCGTAGATTTCTGTTTCTGACCTGCGATCATGGCTTTCTGCTGAAATTCGCGGCAGTACTGCTGA
CGCGTTCTGTTTGTGTAAACGCTGGTAGGT

Oligonucleotide #6 (SEQ ID:14)

CGTCTCTCGTCCTGGTCCGAATTCAGATAAGCTTATTTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC
GGCTGACGGCACGCGGTGCCGTAGATTTCTGTTTCTGAC

C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCCCTGGTGGGTTGCGCCAGAACT
TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTTCGGTAA
AGGCTCTGGTCGTGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT
CAGAGCATGGACCAGCAGGATAAAAATCAAACCTGAACCACTCTCTGGAAAAAGTGAAAGCCGGCCAGGTTA
CTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTTCGCACCTACCAGCGTTACAA
CAAACAGGAACGCCGTGACGAGTACTGCCGCGAATTTTCAGCAGAAAGCCATGATCGCAGGTGAGAAACAG
GAAATCTACGGCACCGGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

FIGURE 5

A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSLI I I SVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKSGSRVSM AIGGAVLGGLIGSKIG
QSM DQQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQ
EIYGTACPQPDGRWQVISTEK

B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

ATGTCAGTTGAATTCTACAACCTCTAACAATCAGCACAAACAACTCAATTACACCAATAATCAAAATTA
CTAACACATCTGACAGTGATTTAAATTTAAATGACGTAAAAGTTAGATATTATTACACAAGTGATGGTAC
ACAAGGACAACTTTCTGGTGTGACCATGCTGGTGCAATTATTAGGAAATAGCTATGTTGATAACACTAGC
AAAGTGACAGCAAACTTCGTTAAAGAAACAGCAAGCCCCAACATCAACCTATGATACATATCTGGATCCGT
CTCATATGCGTGGTTGCCGTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCCTGGTGGGTTGCGCCA
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTT
GGTAAAGGCTCTGGTCTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCAAGTCTCTGGAAAAAGTGAAAGCCGCCA
GGTTACTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTTCGCACCTACCAGCGT
TACAACAAACAGGAACGCCGTCAGCAGTACTGCCGCGAATTTTCAGCAGAAAGCCATGATCGCAGGTCAGA
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

MSVEFYNSNKS AQTNSITPI I KITNTSDSDLNLNDVKVRYYYTSDGTQGQTFWCDHAGALLGNSYVDNTS
KVTANFVKETASPTSTYDTYLDPSHMRGCLQGSSLI I I SVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF
GKSGSRVSM AIGGAVLGGLIGSKIGQSM DQQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQR
YNKQERRQQYCREFQQKAMIAGQKQEIYGTACPQPDGRWQVISTEK

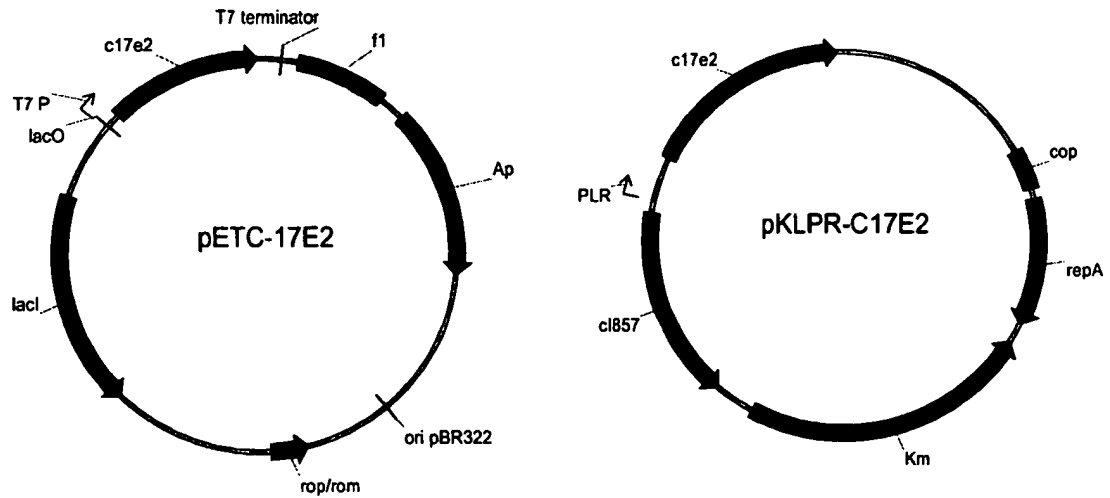
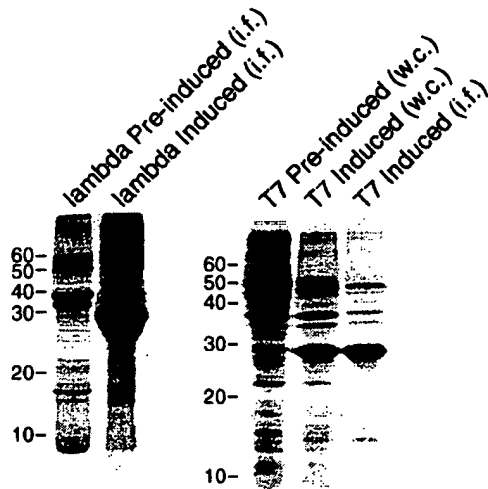
FIGURE 6**A. Expression vectors encoding the optimized *ospA* fusion constructs****B. SDS-PAGE analysis of C17E2 expression.**

FIGURE 7

Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.

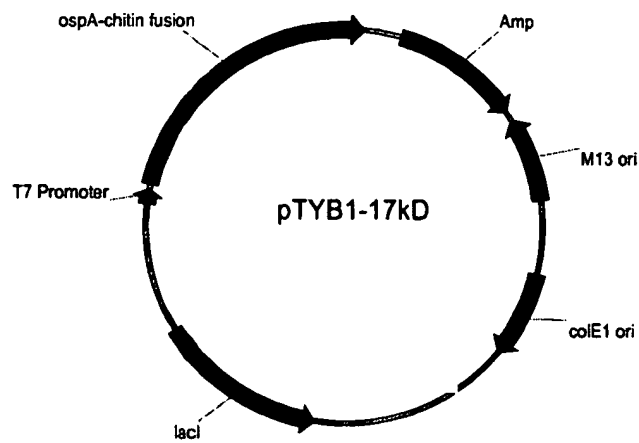
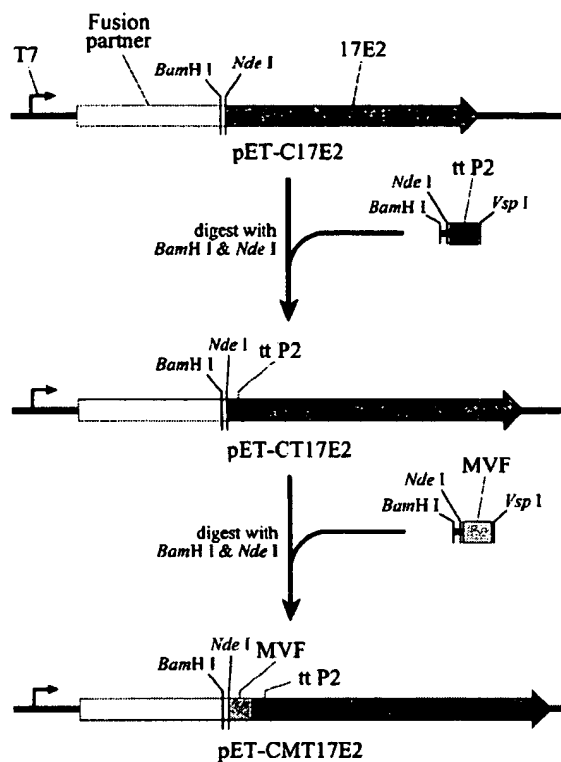


FIGURE 8

A. CLONING STRATEGY FOR OSPA TCE FUSION PROTEIN CONSTRUCTS.



B. (a) Nucleotide sequence of the tt P2 oligonucleotide (SEQ ID:17)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCAGTACATTAAAGCAAACCTCTAAATTCATC
GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCTGTCTGAAATCAAAGGTGTTATCGTTCAT
CGTCTGGAAGGCGTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(c) Amino acid sequence of the tt P2 TCE (SEQ ID:19)

QYIKANSKFIGITEL

(d) Amino acid sequence of the MVF TCE (SEQ ID:20)

LSEIKGVIVHRLEGV

FIGURE 9

Coho salmon antibody titres against OspA-fusion protein candidate vaccines.

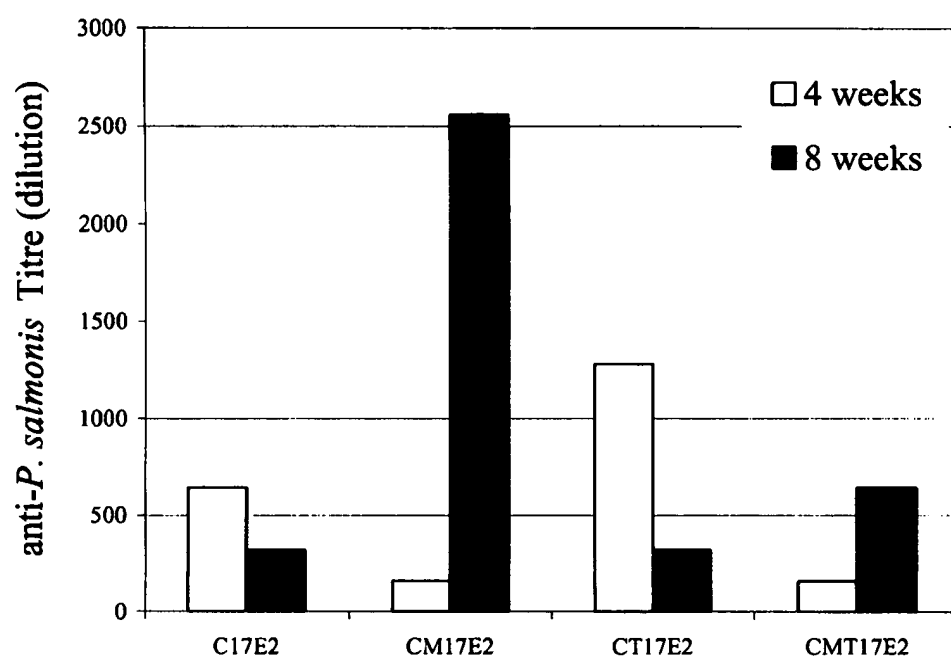


FIGURE 10

Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.

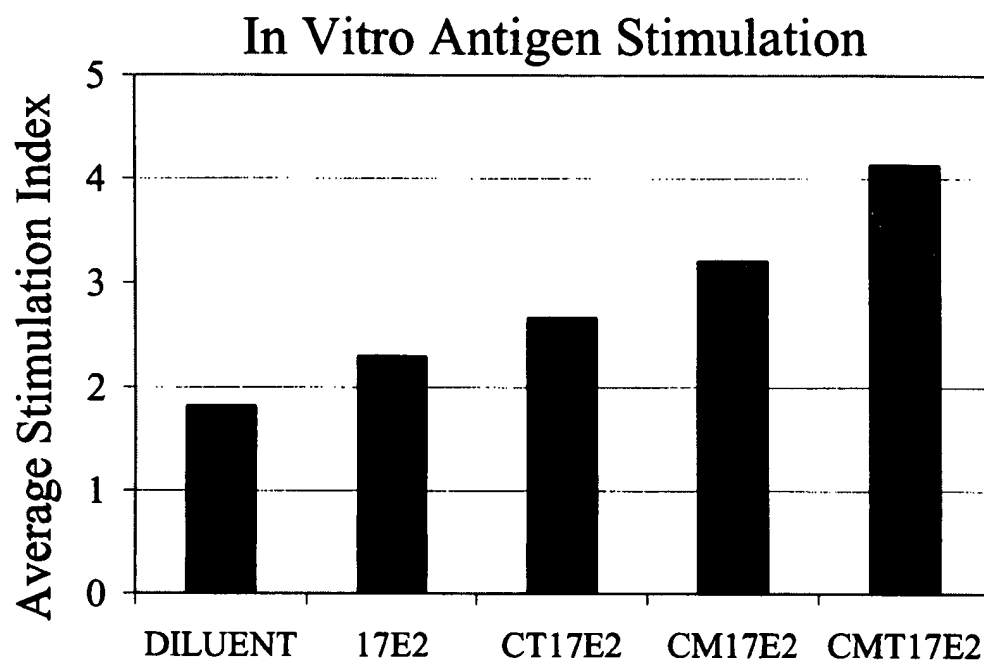


FIGURE 11**Vaccine trial in coho salmon of OspA fusion proteins.**